

1 BIODEGRADABLE BLOCK COPOLYMERS
2 WITH MODIFIABLE SURFACE

3
4 BACKGROUND

5
6 Technical Field

7 The disclosure relates to block copolymers with a hydrophobic biodegradable
8 component and a hydrophilic biocompatible component, which permit the selective
9 binding of surface-modifying substances and at the same time can suppress the non-
10 selective adhesion of unwanted substances, and to shaped bodies produced therefrom.

11 Such block copolymers are particularly suitable as carriers for cells for tissue
12 culture, as carriers for active substances such as medications, in particular for
13 controlled release (drug delivery system) and for targeted administration of active
14 substances (drug targeting).
15

16 Related Art

17 Biomaterials, which include the block copolymers according to the disclosure,
18 play a dominant role in a range of medical applications. The term biomaterials relates
19 to substances which assume a specific function in human or animal body as substitute
20 substances for endogenous materials. Examples of this are metals or polymers, such
21 as, for example, those used in total endoprosthesis in the region of the hip joint. A
22 disadvantage of many biomaterials, which are only used temporarily in the body, such
23 as pins or plates in the surgical field, for example, is that they have to be removed
24 after application. For this reason, at the beginning of the seventies an intensive search
25 was started for biodegradable materials which degrade into fragments tolerated by the
26 body during the application.

27 The term "biodegradable" means that the biological system, into which the
28 material is introduced, contributes to its degradation [Vert, M et al "Degradable
29 Polymers and Plastics" Redwood Press Ltd. (1992) 73-92]. Those particularly worthy

1 of note are biodegradable polymer materials which degrade into oligomers or
2 monomers. Surgical suture material or degradable carriers of medicinal agents are
3 mentioned as examples of their application.

4 The successful application of biodegradable polymers has led to an intensive
5 search for new synthetic materials, from which a plurality of different polymer classes
6 resulted, such as poly(a-hydroxyesters), poly(b-hydroxyesters), polyanhydrides,
7 polycyanoacrylates and many others [Göpferich, A. (1997) 451-472; Göpferich, A.:
8 Biomaterials 17 (1996a) 103-114; Göpferich, A. Eur. J. Pharm. Biopharm. 42 (1996b)
9 1-11].

10 A particular characteristic of these polymers is their low solubility in aqueous
11 media, which only improves through polymer chain degradation, i.e. hydrolysis to
12 lower-molecular oligomers or monomers, and thus leads to erosion of these materials.

13 Besides the development of synthetic biodegradable polymers, an intensive
14 search was instigated at the same time for natural polymers, which have similar
15 properties. Examples of these are collagens, hyaluronic acid, alginate and cellulose
16 derivatives [Park, K. et al: Biodegradable Hydrogels for Drug Delivery (1993)]. With
17 these substances, it is accepted to some extent that they have an increased water
18 solubility. To lower the water solubility, natural polymers are often chemically
19 modified, e.g. by etherification and esterification of functional groups in the polymer
20 chain or by cross-linkage of individual strands. By way of example, the cross-linkage
21 of collagens, gelatine or alginate are mentioned here.

22 Various biodegradable polymers differ above all by the rate of polymer chain
23 degradation and erosion. This is important for many applications, in which the
24 polymer chain degradation must extend over a defined time period, such as in the case
25 of release of medicinal agents, for example.

26 It is essential for the medicinal application of synthetic, part-synthetic and
27 natural biodegradable polymers that they are compatible with the biological system
28 into which they are introduced. For applications in human or animal organisms.

1 individual structural elements, such as oligomers or monomers, must not be toxic and
2 the polymers may trigger, at most, a moderate inflammatory reaction in the tissue.

3 The above-mentioned biodegradable polymers are currently used for the
4 controlled release of medicinal agents (drug delivery) [Göpferich, A. Eur. J. Pharm.
5 Biopharm. 42 (1996b) 1-11] and as carriers for cells (tissue engineering) [Langer, R
6 and Vacanti, J.P. Science 260 (1993) 920-926].

7 As part of the drug delivery, biodegradable polymers release medicinal agents
8 in a controlled manner by diffusion, erosion, swelling or osmotic effects.

9 In the field of tissue engineering, biodegradable polymers as used as porous
10 "sponges," for example, on which cells can adhere, proliferate and be differentiated.
11 While the cells develop to a tissue band, the polymer carrier degrades and a tissue
12 results which may be transplanted into the human or animal body.

13 Examples of tissues currently produced in this way are, inter alia, cartilage,
14 bone, fatty tissue and vessels.

15 The application of biodegradable polymers in the fields of tissue engineering
16 and drug delivery set particular requirements for these materials.

17 Besides the already mentioned biocompatibility of the polymers and their
18 degradation products, these applications set particular requirements for the surface
19 properties of the polymers.

20 Some examples from the field of drug delivery shall be named firstly:

21 1. An adsorption of molecules (for example, medicinal agents, proteins and
22 peptides) onto the polymer surfaces is frequently observed. This can result in the
23 biodegradable medicinal agent carrier not releasing its dosage to the desired extent and
24 not with the desired kinetics. In an extreme case, this can also lead to inactivation of
25 the active substance. The adsorption of active substances is therefore undesirable in
26 many cases and must be suppressed.

27 2. The compatibility of a biodegradable polymer is greatly dependent on its
28 surface properties. Hence, these polymers in the form of particles in the micrometer
29 and nanometre range are recognized by cells of the immune system such as

1 macrophages, for example, after absorption of endogenous proteins, and subsequently
2 phagocytised.

3 It is therefore necessary to examine the surface properties of small particles as
4 parenteral forms of medicines for their successful use.

5 3. Biodegradable nanoparticles have long been sought to use for the targeted
6 administration of substances to specific tissue (for example, tumors or central nervous
7 system) (drug targeting). It has been found in this case that endogenous proteins which
8 are adsorbed on the particle surfaces are responsible for where these particles are
9 transported. [Juliano, R.L.: Adv. Drug Delivery Rev.2 (1988) 31-54]. Hitherto it has
10 only been conditionally possible to achieve a targeted adsorption of these proteins
11 onto the particles. Polymers which allow the targeted modification of their surfaces
12 by simple means are therefore advantageous.

13 The surface properties of biodegradable polymers also play an important role
14 in the field of tissue engineering:

15 1. The interactions between cells and polymer determine cell growth and cell
16 differentiation. Natural anchorage mechanisms of the cells are responsible for
17 adhesion of the cells to the polymer surfaces. Proteins such as integrins, for example,
18 allow cells to adhere to specific amino acid sequences. The adhesion to biodegradable
19 polymers occurs as a result of proteins from body fluids or cell culture media
20 adsorbing non-specifically to the polymer surfaces and, in turn, the cells themselves
21 adhering to the corresponding amino acid sequences of the proteins. This non-specific
22 adsorption of proteins causes a plurality of different cells to adhere to the surface. This
23 is above all disadvantageous if a specific cell type is to be adhered to the
24 biodegradable polymer. It is therefore desirable to examine the adsorption of proteins
25 and peptides.

26 2. The amino acid sequences to which cells adhere are often specific for a cell
27 type, i.e. if the surface of a polymer is coated with a cell-specific sequence, then this
28 cell type preferably adheres.

3. The membrane of a cell carries a series of receptors, in which case the behavior of the cell can be influenced via these receptors. Therefore, if corresponding "signal substances" such as hormones, growth factors or cytokines, for example, are located on the surface of polymers, to which the receptors can bind, the behavior of the cells adhering thereto via the receptors may be influenced via these correspondingly coated polymer surfaces.

The above-mentioned examples show the importance of the surface properties of a biodegradable polymer or the importance of the possibility of selective modification of these surfaces for successful application of the polymer. The modification of surface properties of biodegradable polymers has been the aim of intensive research for some years.

The first attempts at producing biodegradable polymers with modifiable surfaces started from incorporating monomers such as lysin, for example, which contain a functional group to which the molecules can adhere, into the polymer chain of poly(α -hydroxyesters), e.g. polylactide, [Barrera, D.A. "Synthesis and Characterization of a Novel Biodegradable Polymer - Poly(lactic acid-co-lysin)" 1993, Massachusetts Institute of Technology, PHD Thesis].

A disadvantage of these polymers is that the functional groups, in this case amino groups, are only accessed in the surface with difficulty. In order to improve this, oligopeptides were adhered to these functional groups in order to facilitate the binding of new chemical bonds.

A disadvantage is that the non-specific adsorption of unwanted proteins and peptides occurs in the polymer obtained.

This led to new developments in order to obtain a more broadly applicable system [Patel, N., Padera, R., Sanders, G.H., Cannizzaro, S.M., Davies, M.C., Langer, R., Roberts, C.J., Tendler, S.J., Williams, P.M. and Shakesheff, K.M. "Spatially controlled Tissue Engineering on Biodegradable Polymer Surfaces." 25(1), 109-110, 1998. Controlled Release Society, Inc. Proceed. Int'l. Symp. Control. Rel. Bioact. Mater. 1998]. In this case the binding of biotin to the protein avidin which is very

1 specific is utilized. Biotin is anchored on a polymer surface and biotin is also bound
2 to the substance with which the surface is to be coated. In the presence of avidin,
3 which has several binding points for biotin, the targeted adhesion of the biotinylated
4 compound to the surface then results.

5 An advantage of the process is that patterns may be generated on the polymer
6 surface. This is important for tissue where a structured arrangement of cells is
7 necessary.

8 However, a disadvantage is that by anchoring avidin, a protein is used which
9 is exogenous and can therefore lead to undesirable reactions. In addition, the substance
10 to be anchored must first be biotinylated, which complicates the process and thus
11 restricts applicability. At the same time, the surface is coated with avidin, which is
12 undesirable for many applications.

13 Other methods use a further polymer to adhere surface-modifying substances
14 to the surface of the biodegradable polymer. Hence, polyethylene glycol is adhered
15 to the surface to be modified, for example, which assumes the corresponding existence
16 of functional groups to the surfaces [U.S. Patent No. 5,908,828]. In these
17 developments, these functional groups must first be generated in some cases by
18 chemical reactions. This is an additional process step and undesirably increases the
19 expense for application of this process.

20 The anchoring of special peptide sequences on ceramics, polyhydroxy ethyl
21 methacrylate and polyethylene terephthalate is described in US Patent 5,330,911. The
22 process assumes the existence of functional groups and is not suitable for the
23 suppression of non-specific adsorption.

24 U.S. Patent No. 5,308,641 discloses a further process is based on
25 polyalkylimine as spacer between the polymer surface and the substance to be
26 adhered. The process has the same disadvantages as described in U.S. Patent No.
27 5,330,911 and assumes the existence of corresponding functional groups on the
28 polymer surface.

1 U.S. Patent No. 5,897,955 and WO 97/46267 A1 disclose a process wherein
2 the surface of the polymer to be modified is firstly coated with a surfactant, which
3 then only after cross-linking forms the actual surface onto which the substances can
4 be bound. The resulting disadvantage here is also that no adequate masking of the
5 surface is achieved and non-specific adsorption cannot be suppressed.

6 To increase the compatibility of polymer surfaces, it has been suggested that
7 asymmetric molecules should be bonded onto these surfaces via radical mechanisms.
8 This procedure is therefore bound to specific materials which firstly adsorb on the
9 polymer surface and can then be cross-linked.

10 According to the US Patent 5,263,992, the surface of biomaterials is firstly
11 covered with a binding molecule in a radical reaction, in which case the binding
12 molecule carries a functional group, onto which surface-modifying substances are
13 bonded. The disadvantage of the process is again that the adsorption of undesirable
14 substances is not suppressed by this structure.

15 US Patent 5,320,840 describes a polymer which is water-soluble and does not
16 therefore meet the requirements for a solid water-insoluble biodegradable matrix.
17 Many processes such as the one described in US Patent 5,240,747, for example,
18 require drastic conditions for the modification of polymer surfaces, e.g. such as
19 radiation with uv light or the presence of functional groups in the form of amino
20 groups or polyamines (US Patent 5,399,665 and US Patent 5,049,403).

21 EP 0 844 269 discloses a block polymer with functional groups at both ends,
22 wherein the block polymer is composed from hydrophobic and hydrophilic blocks.
23 The hydrophilic blocks in this case carry as functional groups amino, carboxyl or
24 mercapto groups, which have to be firstly activated for a covalent linkage of surface-
25 modifying molecules of interest, which generally have amino, mercapto, hydroxyl
26 groups or double bonds as functional groups.

27 WO 95/03356 discloses non-linear block copolymers which are composed
28 from a multifunctional polymer, to which hydrophilic and hydrophobic polymers are
29 bonded. In this case a possible covalent bonding of modifying substances is likewise

1 achieved via a terminal hydroxyl group of the hydrophilic block, e.g. of polyethylene
2 glycol, which requires previous activation.

3 4 SUMMARY

5 The examples outlined above show the need for biodegradable polymers which
6 have the following properties:

- 7 1. Adequate masking of the polymer surface for the suppression of non-
8 specific adsorption of substances;
- 9 2. Suppression of non-specific adhesion of living cells;
- 10 3. Full biodegradability and biocompatibility of the degradation products;
- 11 4. Adjustability of the concentration of functional groups on the polymer
12 surface, which are suitable for the chemical reactions with a plurality of surface-
13 modifying substances;
- 14 5. Provision of the possibility of coating the polymer surface with several
15 different substances;
- 16 6. to permit binding of the surface-modifying substances before or after
17 processing to shaped bodies (e.g. films, porous sponges, microparticles, nanoparticles,
18 micelles etc.), and
- 19 7. Formation of patterns by binding surface-modifying substances on the
20 polymer surface.

21 Two preconditions must be met in order to permanently anchor surface-
22 modifying substances on polymer surfaces:

- 23 1. On their surface the polymers must carry functional groups to which
24 the substances may be chemically bonded.
- 25 2. The functional groups must be readily accessible for these chemical
26 reactions.

27 While known biodegradable polymers such as poly(α -hydroxyesters) [e.g.
28 poly(lactide), poly(lactide-co-glycolide)], polyanhydrides or poly(β -hydroxyesters)
29 have suitable functional groups at both molecule ends, these groups are only accessed

1 on the surface with difficulty. Poly(lactide), for example, has an alcohol and a
2 carboxylic acid function as end group which do not, however, permit binding to the
3 polymer surface for the reasons given above.

4 To achieve the aforementioned objects, a block copolymer is provided
5 according to the disclosure containing

6 a hydrophobic biodegradable polymer a),

7 a hydrophilic biocompatible polymer b),

8 at least one reactive group c) for covalent binding of a surface-modifying
9 substance d) to the hydrophilic polymer b),

10 wherein the at least one reactive group c) is an at least bifunctional molecule
11 with at least one free functional group.

12 According to a further aspect, the disclosure relates to a surface-modified
13 block copolymer which has as additional component a surface-modifying substance
14 d) bonded by means of the reactive group c) as binding link, and a process for the
15 production thereof.

16 In a preferred configuration, the block copolymers are present as shaped
17 bodies.

18 The disclosure further relates to the application of the block copolymers in
19 particular in the field of drug delivery, drug targeting, and preferably for tissue
20 engineering.

21 According to a further aspect the disclosure relates to a process for the
22 production of a block copolymer, wherein the binding of the at least one substance d)
23 to the surface of the block copolymer is achieved by generating a substrate pattern,
24 and the reactive group c) is selected from 1) an at least bifunctional molecule with at
25 least one free functional group and/or 2) a functional group, and block copolymers
26 obtainable with this.

27 Because of their structure comprising a hydrophobic and a hydrophilic
28 component, the block copolymers according to the disclosure have a surfactant-like
29 character. This causes the polymer, e.g. upon contact with an aqueous medium, to be

1 subject to an orientation wherein the hydrophilic component b) is present in enriched
2 form on the polymer surface, and thus allows free accessibility of surface-modifying
3 substances d) to the reactive group c) for binding.

4 Therefore, the disclosure relates to polymers, in which a part of the chain, the
5 hydrophilic component b), projects out of the polymer surface and ensures an adequate
6 distance between the polymer surface and reactive group c), as a result of which the
7 binding of surface-modifying substances to the reactive group c) is facilitated.

8 As a result, special surfaces may be constructed by simple means and prepared
9 for such applications in the best possible way in which the surface of materials serves
10 to assume a specific functionality.

11 At the same time, the block copolymers according to the disclosure ensure
12 suppression of the non-specific adsorption of molecules and adhesion of cells to their
13 surface.

14 An important property of the block copolymers described here is the full
15 biocompatibility of the molecule parts used, in which case at least the hydrophobic
16 component a) is biologically degradable.

17 These polymers also have an advantage in this respect in comparison to
18 systems already described for the modification of surfaces which make use of
19 polystyrene, glass or metals, for example. [Mikulec, L.J. and Puleo, D.A. J. Biomed.
20 Mater. Res. 32 (1996) 203-208; Puleo, D.A. J. Biomed. Mater. Res. 29 (1995) 951-
21 957; Puleo, D.A. Biomaterials 17 (1996) 217-222; Puleo, D.A. J. Biomed. Mater. Res.
22 37 (1997) 222-228).

23 In contrast to the named materials, after implantation into the human or animal
24 body, the block copolymers according to the disclosure have the potential to degrade
25 in a specific period of time, depending on the requirement, and to leave the body.

26 The material properties of the block copolymer can be fixed by the selection
27 of components a) and b) of the block copolymer, i.e. the type and length of the
28 hydrophobic and the hydrophilic polymer chain. For example, the mobility of the
29 fixed substance d) can be varied via the length or structure of the hydrophilic

1 component b). The degradation properties, the mechanical strength and the solubility,
2 for example, in water or an organic solvent of the copolymer can be controlled via the
3 length and structure of the hydrophobic component a).

4 Hence, by changing the biodegradable lipophilic chain of component a) of the
5 block copolymer, it is possible to increase the period of degradation and increase the
6 mechanical strength of the polymers.

7 The configuration as block copolymer according to the disclosure supports the
8 orientation, wherein the hydrophilic component predominantly comes to lie on the
9 polymer surface and, for example, promotes the formation of micelles in the aqueous
10 medium.

11 12 BRIEF DESCRIPTION OF THE DRAWINGS

13 In the drawings:

14 FIG. 1 shows the binding of a surface-modifying substance d) onto the surface
15 of a block copolymer according to the disclosure via the reactive group c);

16 FIG. 2 shows the structure of a block copolymer according to the disclosure;

17 FIG. 3 shows a surface of a block copolymer according to the disclosure coated
18 with different substances d);

19 FIG. 4 shows images taken by scanning microscope of block copolymers
20 according to the disclosure containing different amounts of polyethylene glycol with
21 a molecular weight of 5000 Da and a reference polymer with no PEG;

22 FIG. 5 shows ESCA spectra of protein adsorption on different polymer films;

23 FIG. 6 shows ESCA spectra of peptide adsorption on different polymer films;

24 FIG. 7 shows images taken by optical microscope of pre-adipocytes 3T3-L1
25 on different polymer films;

26 FIG. 8 shows REM images of mesenchymal stem cells from rats on different
27 polymers;

28 FIG. 9 shows determination of the activity of a block copolymer
29 according to the disclosure via the binding of EDANS, and

1 FIG. 10 shows the binding of trypsin to a polymer according to the disclosure.

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3 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

4 The subscript indexes used in the polymer designations in the FIGS. relate to
5 the molecular weight (Mn) expressed in kDa.

6 FIG. 2 shows a surface-modified block copolymer according to the disclosure
7 with its essential structural elements, hydrophobic component a), hydrophilic
8 component b) and reactive group c) as well as surface-modifying substance d).

9 In this case, the hydrophobic component a) serves as carrier and for fixing the
10 entire block copolymer, the hydrophilic component b) serves to make available the
11 reactive group c) for the covalent binding of a surface-modifying substance d) and for
12 masking the surface, and the reactive group c) serves as binding link for the permanent
13 binding of the surface-modifying substance d).

14 The block copolymer according to the disclosure can be brought into any
15 desired suitable shape for the respective applications, the shaped bodies obtained in
16 this case likewise being subject of the disclosure.

17 The block copolymer can, for example, be provided as a film, particle in the
18 desired size, e.g. nano- or micro-particle, or three-dimensional shaped body, e.g.
19 monolith. The shaped bodies can be porous. According to a preferred embodiment, the
20 block copolymer forms a porous shaped body in the manner of a sponge, for example.

21 It is advantageous according to the disclosure that the block copolymer or the
22 shaped bodies formed therefrom are suitable for "instant reactions" with the substance
23 d), which means that they can be produced in advance as stock and stored without
24 problem until application without having to be freshly prepared first for the scheduled
25 application in a time-consuming manner.

26 The block copolymer can be composed from one or more, also different,
27 blocks comprising the hydrophobic a) and hydrophilic component b), in which case
28 the individual blocks can contain the same monomers possibly with different chain
29 lengths, or different monomers.

1 According to a preferred configuration, a diblock copolymer is used as block
2 copolymer.

3 Components a) and b), simultaneously or independently of one another, can
4 be linear or branched, comb- or star-shaped.

5 Component c) can also be a cross-linked compound, if required.

6 The surface of the block copolymer can be coated with a single substance or
7 different substances d), the at least one substance d) can form any desired pattern on
8 the surface, e.g. the concentration of the at least one substance d) can be locally
9 constant or variable, it can form a gradient etc.

10 The type of coating of the surface can be selected in accordance with the
11 application case. Hence, it has been shown that a gradual coating with growth factors
12 can be advantageous.

13 Any biodegradable hydrophobic polymer known for the named applications
14 can be used as biodegradable hydrophobic component a), like those which have
15 already been specified above. Further polymers can be derived from the literature.

16 The polymer for component b) can be of synthetic, part-synthetic or natural
17 origin.

18 They can be poly(a-hydroxyesters, e.g. polylactic acid, polyglycolic acid and
19 their copolymers), poly(e-caprolactam), poly(b-hydroxyesters (e.g. poly(b-
20 hydroxybutyrate), poly(b-hydroxy valerate)), poly(dioxanon), polymalic acid,
21 polytartaric acid, polyorthoester, polycarbonate, polyamide, polyanhydride,
22 polyphosphazene, peptide, polysaccharide, protein and other polymers such as those
23 described in Göpferich A. "Mechanism of Polymer Degradation and Elimination" in:
24 Domb A, Kost J, Wiseman D, eds. Handbook of Biodegradable Polymers. Harwood
25 acad. publ. Inc., 1997: 451-472; Göpferich A: "Mechanisms of Polymer Degradation
26 and Erosion" Biomaterials 17 1996a pp. 103-114 and Göpferich A: Biomaterials 17
27 (1996a) 103-114; Göpferich A., Eur. J. Pharm. Biopharm. 42 (1996b) 1-11; Leenslag,
28 J.W. et al Biomaterials (1987) 311-314; Park, K et al. Biodegradable Hydrogels for
29 Drug Delivery (1993); Suggs, L.J. and Mikos, A.G. (1996) 616-624.

1 Further suitable compounds are described, for example, in the Handbook of
2 Biodegradable Polymers (1997) 451-472.

3 The hydrophobic polymer a) is preferably at least one polymer selected from
4 a polyester, poly-e-caprolactam, poly-a-hydroxyester, poly-b-hydroxyester,
5 polyanhydride, polyamide, polyphosphazene, polydioxanon, polymalic acid,
6 polytartaric acid, polyorthoester, polycarbonate, polysaccharide, peptide and protein.

7 The hydrophobic polymer a) is, in particular, at least one polymer selected
8 from polylactide, polyglycolide, poly(lactide-co-glycolide), poly-b-hydroxybutyrate
9 and poly-b-hydroxyvalerate.

10 The hydrophobic component a) is preferably water-insoluble.

11 The polymers particularly suited as biodegradable component a) are those in
12 which the polymer chain degradation can be brought about by hydrolysis, enzymatic,
13 photolytic or other reactions.

14 The minimum chain length n measured in monomers amounts to $n=2$, the
15 upper limit results from the maximum achievable molar masses for the respective
16 monomer in the polymerisation reaction or from the desired properties for the
17 polymer, i.e. depending on the intended application.

18 As part of the present disclosure the details concerning the molar masses
19 (molecular weight), unless specified otherwise, relate to the numerical mean M_n .

20 Hence, the chain length of the polymers for component a) can move from few
21 to several thousand monomer units and the polymer can have a molecular weight of
22 over 10 million Dalton.

23 For example, for polylactide an upper limit of the molar mass of up to 100 000
24 Da is preferred.

25 As already mentioned above, the length of the hydrophobic component a)
26 determines the properties of the block copolymer such as the degradation properties
27 and the mechanical strength.

28 For example, in the case of a combination preferred according to the disclosure
29 of poly(D,L-lactide) (PLA) as hydrophobic component a) and poly(ethylene glycol)

1 (PEG) for the hydrophilic component b), a chain length of the hydrophobic component
2 a) of approx. $n < 20$ leads to water-soluble products. If the PEG content is greater than
3 the PLA content, then water-soluble products can likewise be expected.

4 A synthetic, part-synthetic or natural biocompatible hydrophilic polymer,
5 which can also be biologically degradable, may be used as hydrophilic component b).

6 It is built up from at least bifunctional and preferably water-soluble structural
7 elements.

8 Examples of suitable polymers are polyethylene glycols, polyacrylamides,
9 polyvinyl alcohol, polysaccharides (e.g. modified celluloses and starches), alginates,
10 peptides and proteins.

11 Preferred examples for the hydrophilic component b) are polyethylene glycol,
12 polypropylene glycol, polyethylene glycol/polypropylene glycol copolymer,
13 polyethylene glycol/polypropylene glycol/polyethylene glycol copolymer,
14 polybutylene glycol, polyacrylamide, polyvinyl alcohol, polysaccharide, peptide and
15 protein.

16 If a symmetric molecule such as PEG, for example, with two like functional
17 end groups, in this case hydroxyl, is used as hydrophilic component b), it should be
18 ensured during linkage with the hydrophobic component a) that the hydrophobic
19 component does not react with both functional end groups simultaneously, and thus
20 none of the functional end groups remains available as reactive group c) for the
21 covalent binding of surface-modifying substances.

22 To avoid this problem, a hydrophilic component b) with two different
23 functional end groups is used for the synthesis, as will be explained below by the
24 example of the preferably used PEG, in which case these explanations apply
25 analogously for other symmetric molecules which may be used as hydrophilic
26 component b) for the block copolymer according to the disclosure. Thus, in the case
27 of PEG with two hydroxyl groups as end groups, one of the hydroxyl groups is
28 replaced by another functional group.

1 For example, poly(ethylene glycol) amine (PEG-NH₂) may be used, in which
2 case an end hydroxyl group is replaced by a primary amino group.

3 This permits the adhesion of the monomers of the hydrophobic component a)
4 to be controlled as part of the synthesis in such a way that the chemical reaction only
5 proceeds at one molecule end.

6 The type of functional end groups is not restricted in this case to hydroxyl
7 groups and amino groups. Alternatively, thiol groups, double bonds or carbonyl
8 functions may be used for synthesis. Further functional groups are known per se and
9 can be derived from the literature.

10 The chain length of the hydrophilic component is also determined in
11 accordance with the application and requirement.

12 For example, the minimum chain length for PEG or of an asymmetric
13 substituted PEG such as PEG-NH₂, for example, is at an ethylene unit (ethanolamine).

14 The upper limit can be set for specific applications in human and animal
15 bodies by the requirement that the released fragments should still be capable of
16 passing through the kidneys and can be excreted.

17 Suitable molar masses preferably lie at 200 to 10,000 Da, particularly preferred
18 at 1,000 to 10,000 Da, in which case, in particular for applications outside a human
19 or animal body, polymers with higher molar masses of up to several million Da may
20 also be used.

21 Above all, PEG has proved to be particularly suitable to masking a polymer
22 surface against the adsorption of molecules and the adhesion of cells.

23 Block copolymers composed from the following combinations are particularly
24 preferred according to the disclosure.

25 The hydrophobic polymer a) is at least one selected from polylactide,
26 polyglycolide, poly(lactide-co-glycolide). Particularly preferred is a polylactide, e.g.
27 a poly(D,L-lactide), preferably with a molar mass in a range from 1,000 to 100,000,
28 in particular up to 50,000 Da.

1 The hydrophilic polymer b) is a polyethylene glycol (PEG), wherein
2 polyethylene glycols with a molar mass in a range from 200 to 10,000 Da, in
3 particular 1,000 to 10,000 Da, are particularly preferred.

4 In principle, the reactive group c) can be any desired functional group or an at
5 least bifunctional molecule, which can form a covalent bond with the selected surface-
6 modifying substance d), with the provision that an at least bifunctional molecule is
7 used as reactive group c) for a block copolymer according to one of Claims 1 to 19.
8 The reactive group c) can comprise:

9 a single functional group (e.g. amino group, carboxyl group) and thus direct
10 activation of the hydrophilic polymer (e.g. activated acid function or epoxide);

11 physiological dicarboxylic acids (succinic acid, tartaric acid and variants
12 thereof such as those described in Anderson, G.W. et al. J.Am.Chem.Soc.86 (1964)
13 1839-1842), which are provided with terminal groups (succinimidyl esters) in order
14 to achieve the formation of one or two acid amide groupings;

15 dialdehydes (e.g. glutaric dialdehyde);

16 special "molecules" for the selective binding of thiols such as those described
17 in Hermanson, G.T. Bioconjugate Techniques (1996), e.g. N-succinimidyl-3-(2-
18 pyridyldithio)propionate (SPDP) or succinimidyl-4-(N-maleimidomethyl)-
19 cyclohexane-1-carboxylate (SMCC);

20 photoreactive crosslinkers such as those described in Hermanson, G.T.
21 Bioconjugate Techniques (1996), e.g. N-hydroxysuccinimidyl-4-acidosalicylic acid
22 (NHS-ASA), sulphosuccinimidyl-2-(p-acidosalicylic amido)ethyl-1,3'-
23 dithiopropionate (SASD);

24 splittable crosslinkers such as those described in Hermanson, G.T.
25 Bioconjugate Techniques (1996), e.g. compounds from the above-mentioned groups,
26 which may be split by special reagents e.g. disulphides by hydrogenolysis or by
27 disulphide exchange, glycol groups with periodate (e.g. in the case of tartaric acid),
28 ester groups with hydroxylamine; and

1 enzymatically splittable molecules such as corresponding peptides, e.g. the
2 sequence Leu-Gly-Pro-Ala, which can be split from collagenase, or oligosaccharides.

3 Particularly preferred examples of reactive groups c) are those selected from
4 at least one amino group, hydroxyl group, thiol, carboxylic acid, acid chloride, keto
5 group - and in particular for the subject of Claims 1 to 19 - dicarboxylic acid amide,
6 3-maleic imidopropionic acid-N-succinimidyl ester and succinimidyl ester.

7 In principle, the synthesis of the block copolymer according to the disclosure
8 may be achieved in various ways, in which case conventional methods of polymer
9 chemistry are used.

10 On the one hand, the blocks a) and b) can be synthesized separately and
11 subsequently bonded covalently. Alternatively thereto, it is possible to present a
12 polymer chain and synthesis the missing chain by polymerisation at a polymer chain
13 end. Hence, it is possible, for example, to synthesize block copolymers from
14 poly(D,L-lactide) and poly(ethylene glycol) amine (PLA-PEG-NH₂) by presenting
15 PEG-NH₂ and synthesizing the biodegradable PLA chain by ring-opening
16 polymerisation from dilactide on the hydroxy end of the PEG-NH₂. In principle, the
17 reverse procedure is also possible.

18 In this case, the reactive group c) can already be present in the polymer
19 obtained, as in the above example, or a functional group present in the hydrophilic
20 component b) can be converted or introduced, where needed, for binding the desired
21 surface-modifying substance d) to a suitable reactive group c).

22 Hence, the block copolymer can be modified with nucleophilic groups by
23 coupling an at least bifunctional molecule, e.g. disuccinimidyl succinate, to a free end
24 group of component b).

25 In the simplest case, this reaction can take place in solution, DMSO, for
26 example, is suitable as solvent in the case of PLA-NH₂. After preparation of the block
27 copolymer, e.g. to form a suitable shaped body, the reaction can also take place on the
28 surface thereof.

1 The advantage of activation with a reactive group c) is that the linking of many
2 surface-modifying substances d) proceeds in water. As a result of the reactive group
3 c), which is linked to the hydrophilic block b), this block ends with an active group,
4 which is capable of binding other molecules with nucleophilic functional groups, such
5 as amino groups, for example. FIG. 1 schematically shows the adhesion of a surface-
6 modifying substance to such a polymer surface.

7 The desired surface property can then be set via the subsequently occurring
8 adhesion of the surface-modifying substance d) to the hydrophilic molecule part b).

9 Surface-modifying substances d), which may be used for a bond, are generally
10 those carrying a nucleophilic group - e.g. an amino group -, such as carbohydrates, for
11 example, including amongst others: mono-, oligo-, and polysaccharides and
12 glycosides, peptides, proteins, heteroglycans, proteoglycans, glycoproteins, amino
13 acids, fats, phospholipids, glycolipids, lipoproteins, medicinal agents, antibodies,
14 enzymes, DNA/RNA, cells, which can bond directly, for example, via proteins located
15 on the cell membrane, but also dyes and molecular sensors.

16 Examples for peptides are those with the motif -RGD-, IKVAV or YIGSR and
17 for proteins growth factors, e.g. IGF, EGF, TGF, BMP and basic FGF, proteins and
18 glycoproteins of the extracellular matrix such as fibronectin, collagen, laminin, bone
19 sialo protein and hyaluronic acid. Further substances are described in the relevant
20 literature.

21 The block copolymer according to the disclosure is particularly suitable for the
22 production of drug targeting systems, drug delivery systems, bioreactors, preferably
23 porous shaped bodies, for therapeutic and diagnostic purposes, for tissue engineering
24 and as emulsifier.

25 The binding of the surface-modifying substance is explained in more detail
26 below, in general terms and with respect to preferred applications.

27 For the binding, the block copolymer, like the substance, can be present in
28 solution or the block copolymer forms an immobilized solid surface, to which binds
29 the substance d) present in solution.

1 In this case, a decisive advantage of the use of the block copolymer according
2 to the disclosure is that under very mild conditions the linking reactions may also be
3 conducted in aqueous medium and therefore sensitive substances d) may also be
4 bonded in.

5 Hence, proteins can be fixed at room temperature and with a pH suitable for
6 the protein without being denatured on the polymer surface. Alternatively, substances,
7 which are to be bonded to the surface by means of light radiation, can be dissolved in
8 any desired solvent in which the polymer is insoluble. Upon subsequent radiation with
9 uv light, the binding to the surface can then also be linked at room temperature.

10 Therefore, several conditions are conceivable, in principle, for a binding
11 process, wherein by using the block copolymer according to the disclosure there is
12 sufficient freedom to select optimum conditions with respect to the stability of the
13 substance d) and the polymer.

14 As a result of the simple type of binding of also unchanged, i.e. non-activated
15 substances d), to the block copolymer with reactive group c) made possible according
16 to the disclosure, the process can be simplified insofar as it is only necessary to dip the
17 finished preshaped polymer carrier, e.g. in the form of micelles, nano-particles,
18 polymer film or polymer sponge, into the solution of substance d) in order to then
19 obtain the finished modified system after a predetermined reaction period (instant
20 reaction).

21 However, alternatively to the described binding of substance d) to the polymer
22 with reactive group c), the other way round is also possible, namely to first activate
23 the substance d) to be bound with the reactive substance c) for a bond, and then bind
24 the complex comprising substance d) and reactive group c) via the reactive group c)
25 to the component b) of the block copolymer comprising a) and b) to form the finished
26 surface-modified block copolymer according to the disclosure.

27 However, a disadvantage in this case is that a larger excess of the reactive
28 group c), e.g. a low-molecular dicarboxylic acid here, is generally necessary for
29 activation of the substance d) by binding the reactive group c) in order to prevent the
30 formation of dimers. However, this must be removed again after activation. The

1 consequence of this is, above all with likewise low-molecular substances d), that the
2 purification is more difficult to configure.

3 In addition to the production of homogeneously coated surfaces, non-
4 homogeneously coated surfaces may also be easily produced with the block
5 copolymers according to the disclosure. This means that, for example, gradients or
6 patterns of the surface-modifying substances d) can also be generated on these
7 polymers. This can be achieved by spot application of the substances d) (e.g. using an
8 ink jet process) or by spot activation of the reactive groups c) by radiation (e.g. with
9 uv light), bombardment with particles, stamping or soft lithography.

10 Hence, structured surfaces can be formed which also allow any desired
11 combinations of substances d) to be examined for their effect on cells, for example,
12 or to cultivate combinations of cells in very special spatial orientation to one another
13 or also to construct miniature biotechnological factories using enzymes which perform
14 special reactions in a linked process. FIG. 3 shows such surfaces which are
15 distinguished by two different substances d) and additionally also an inert shorter
16 component.

17 As part of tissue engineering, it is possible to influence the adhesion,
18 proliferation and differentiation of cells in a better way than previously, since the
19 block copolymers according to the disclosure enable an exact coating of the surface
20 with one or more substances d). At the same time, the non-specific interaction of
21 unwanted substances d), in particular unwanted cells, is suppressed with the polymer
22 surfaces.

23 As part of drug delivery, it is possible to use the polymers for surface
24 modification, which distributes small polymer particles to specific tissues or organs
25 (drug targeting). This is achieved by binding specific substances d) such as plasma
26 proteins, antibodies or lectins, for example. Further substances d) possible for this are
27 described in the relevant literature.

28 A further application lies in the chemical bonding of polymers in the form of
29 particles to tissue (bioadhesive systems). An active substance can be distributed in
30 increased concentration to the target tissue by this application.

1 As a result of the polymer degradation it is to be expected that the substance
2 d) adhered to the polymer block b) is released as part of the hydrolysis. This dynamic
3 process permits the time controlled change of the surface properties of the block
4 copolymer according to the disclosure.

5 The polymers according to the disclosure may also be used for diagnostic
6 purposes by binding substances d) to their surface, which form a bond with the
7 molecules to be analyzed. The analyzed product can then be separated from the
8 sample together with the polymer (e.g. via a suitable shaped body).

9 The production of a block copolymer according to the disclosure as well as the
10 subsequent binding of a protein is illustrated below using the example of PEG-PLA
11 to explain the disclosure in more detail.

12 WORKING EXAMPLES

13 EXAMPLE 1: PRODUCTION OF NH₂ PEG-PLA

14
15
16 a) Synthesis of NH₂-PEG. Production was conducted in accordance with
17 Yokohama, M. et al. Bioconj. Chem. 3 (1992) 275-276.

18 The desired amount of ethylene oxide was passed into dry THF in a three-
19 necked flask at -79°C (dry ice + methanol bath) and dissolved therein. The ethylene
20 oxide bottle was weighed after introduction, and thus the presented amount of
21 ethylene oxide was determined. In accordance with the desired molecular weight of
22 the polymer, the calculated amount of 0.5M solution of potassium-bis-(trimethylsilyl)
23 amide in toluene was then added from a dropping funnel.

24 The reaction mixture was then stirred in the closed three-necked flask at 20°C
25 for 36 hours. The polymer solution thus obtained was dropped into the 12-fold amount
26 of ether, and the precipitated polymer was filtered out. After the polymer obtained was
27 dissolved in THF, a small amount of 0.1N hydrochloric acid was added and the
28 silylamide was thus split. The solution of the finished end thus obtained was stirred

1 for 5 minutes at room temperature and once again passed into ether in order to
2 precipitate the pure polymer.

3 b) Synthesis of NH_2 -PEG-PLA. Synthesis was conducted in accordance with
4 Kricheldorf, H.R. and Kreiser-Saunders, I. *Macromol. Symp.* 103 (1996) 85-102;
5 Leenslag, J.W. and Pennings, A.J. *Makromol. Chem.* 188 (1987) 1809-1814.

6 The starting products of the synthesis: the NH_2 -PEG synthesized in accordance
7 with 1a) and cyclic DL-dilactide (3,6-dimethyl-1,4-dioxan-2,5-dion), were each passed
8 into a round flask in the desired weight proportions and dissolved in A.R.toluene. For
9 this, the two flasks were heated at the water separator in order to remove the water still
10 present in the toluene. The solutions thus obtained were then combined in the three-
11 necked flask and once again heated in a permanent nitrogen flow.

12 The weighed catalyst (tin-2-ethylhexanoate) was then added to the boiling
13 reaction mixture and the mixture was then kept boiling for 8 hours.

14 The polymer solution thus obtained was passed into a round flask after cooling
15 and rotated three times with dichloromethane in the rotary evaporator until dry. After
16 rotating twice after the addition of acetone, the polymer thus obtained was once again
17 dissolved in acetone and dropped into ice-cooled demineralized water and precipitated
18 thereby. The polymer threads thus obtained were separated through a filter and passed
19 into a vacuum drying cupboard. Determination of the molecular mass can be
20 performed by GPC.

21 c) Synthesis of the disuccinimidylester of tartaric acid (DSWS). Synthesis was
22 conducted in accordance with Anderson, G.W. et al. *J. Am. Chem. Soc.* 85 (1964)
23 1839-1842.

24 The calculated amounts of tartaric acid and N-hydroxy succinimide were
25 dissolved in a round flask in a mixture comprising dioxan and ethyl acetate (4:1). To
26 this solution the solution of the catalyst (dicyclohexylcarbodiimide) was added in the
27 same solvent mixture and the whole was stirred in an ice bath at 0°C for 20 hours.
28 The precipitate thus obtained was filtered off and washed with dioxan. The end
29 product was extracted from this precipitate by careful heating with acetonitrile. The

1 solution thus obtained was concentrated to low volume in the rotary evaporator and
2 the product dried in the vacuum cupboard.

3 d) Synthesis of SWS-NH-PEG-PLA. The starting products obtained in
4 accordance with 1c) and 1b): disuccinimidyl tartaric acid and NH_2 -PEG-PLA, were
5 dissolved in acetonitrile with a slight excess of the diester and provided with a few
6 drops of triethylamine. After brief heating to boiling, the mixture was stirred for 24
7 hours. The end product was separated from the acetonitrile by rotation and dissolved
8 in acetone. The polymer solution thus obtained was dropped into water and the
9 precipitate filtered off. The finished active polymer was available after drying in the
10 vacuum.

11 According to the above-described procedure NH_2 -PEG-PLA diblock
12 copolymers according to the disclosure were produced with different molecular
13 masses for the components a) and b) for the subsequent experiments or polymers
14 inactivated analogously with methyl groups, in which the reactive group c) was
15 replaced by a methyl group.

17 EXAMPLE 2

18 Production of amino-polyethylene glycol-poly-L-lactide (NH_2 -PEG-PLLA)

19 The procedure was essentially as in Example 1b). However, cyclic L-dilactide
20 was used instead of the cyclic D,L-dilactide. Further, after rotation three times with
21 dichloromethane, the polymer obtained was once again dissolved in dichloromethane
22 and dropped into ice-cooled diethylether. The polymer thread thus obtained were
23 separated through a filter and passed into a vacuum drying cupboard for drying.

24 Determination of the molecular weight was achieved by GC and determination
25 of the numerical mean molecular weight was also achieved by ^1H -NMR via
26 calculation of the integrals.

28 EXAMPLE 3

29 Linkage of surface-modifying substances d).

1 Binding of surface-modifying substances can be conducted in accordance with
2 the processes described in Hill, M. et al. FEBS Lett. 102 (1979) 282-286; Schulman,
3 L.H. et al. Nucleic Acids Res. 9 (1981) 1203-1217.

4 The linkage of surface-modifying substances d) to the block copolymer
5 according to the disclosure obtained in accordance with Example 1 can occur in two
6 ways, in principle. Firstly, it is possible to bind the substance d) and the block
7 copolymer in solution if the substance d) passes through the subsequent processing
8 steps undamaged. Alternatively, the block copolymer may firstly be processed to the
9 desired form and the substance d) is then linked. In both cases, it should be assured by
10 buffering that an amino group, for example, is present in unprotonated form in order
11 to obtain quantitative yields where possible. Moreover, with buffering the location
12 of the bond to the substance d) can still be controlled if the pH is selected so that only
13 an amino group is present in unprotonated form, for example.

14 15 EXAMPLE 4

16 Characterization of polymer films - properties of the block copolymers.

17 4a) Examination of the block copolymers with AFM Scanning microscopy was
18 used to characterize the surface topography of the block copolymers according to the
19 disclosure. For this, the polymers were applied in a 5% solution in chloroform to
20 small square metal plates (5x5 mm) by means of spincoating and then dried. The films
21 thus obtained were then examined with AFM.

22 The results are shown in FIG. 4. What are obtained are different
23 concentrations, depending on the polymer examined, of humped raised portions on the
24 polymer surface. The raised portions are crystallites of the polyethylene glycol which
25 increase with the increasing content of polyethylene glycol in the block copolymer.
26 This means that the polymers are distinguished by a phase separation of the blocks and
27 thus an availability of the hydrophilic chains on the polymer surface.

28 4b) Examination of the protein adsorption

29 Examination of the protein adsorption and its suppression was conducted on
30 different PEG-PLA block copolymers according to the disclosure, which contained a

1 methyl group in place of a reactive group c) and were thus inactivated for the protein
2 bonding.

3 For examination of the adsorption of proteins onto the polymer films such
4 inactive polymers were poured out onto small metal plates (0.5x.05 mm) and
5 intensively dried (for at least 2 days in a vacuum), the films thus obtained were then
6 incubated with the protein solutions to be examined and washed off after washing
7 several times with phosphate-buffered (pH=7.4) of isotonic solution. The films thus
8 obtained were then dried again and measured with ESCA.

9 The model substances were foetal cow serum, atrial natriuretic peptide and
10 salmon calcitonin.

11 The ESCA spectra served to quantify the adsorbed protein or peptide, since
12 nitrogen was also to be found on the polymer surface as a result of the amino acids of
13 the adsorbed protein. As comparison, polymer films from pure polylactic acid as well
14 as non-incubated polymer films were used. The results are shown in FIGS. 5 and 6.

15 A suppression of the adsorption dependent on the type of surface-modifying
16 substance d) respectively used was observed. Hence, the adsorption of foetal cow
17 serum was completely suppressed by inclusion of a hydrophilic chain as part of the
18 measurement accuracy (see FIG. 5). In the case of the model peptides calcitonin and
19 atrial natriuretic peptide (ANP), a low adsorption of peptide is still identifiable in part
20 (see FIG. 6).

21 Therefore, it was established in the result that the block copolymers according
22 to the disclosure are able to control the adsorption of proteins and peptides and can
23 therefore have influence on the behavior of cells which come into contact with the
24 modified polymer surface.

25

26 EXAMPLE 5

27 Examination of the adhesion behavior with respect to cells.

28 5a) Cells from a pre-adipocyte cell line were put in a suspension on poured films
29 made of different polymers and their adhesion assessed after 5 hours and 24 hours.

1 For this, the suspensions were washed off with buffer prior to microscopy, and thus
2 only the firmly adhered cells were observed.

3 The results are shown in FIG. 7. What is evident are differences in the cell
4 behavior dependent on which polymers were used. Hence, for example, on the
5 MePEG₃PLA₂₀ no adhered cells can be recognized both after 5 hours and 24 hours, in
6 which case cells are evident on a small scale on the block copolymer MePEG₃PLA₂₀
7 with the shorter PEG chain, however these adhered only poorly in comparison to the
8 sample composed of lipophilic polylactic acid. After 5 hours only loosely bonded cell
9 aggregates were found and only after 24 hours were single instances of already spread,
10 i.e. firmly bonded, cells found. However, it can be established in the result that the
11 block copolymers according to the disclosure can suppress or reduce the adhesion of
12 cells and can thus prevent or restrict the number of non-specific interactions.

13 5b) For examination of the adhesion of stem cells of rats, thin polymer films made
14 of different block copolymers according to the disclosure inactivated with methyl (Me-
15 PEG₂-PLA₂₀, Me-PEG₂-PLA₄₀ and Me-PEG₃-PLA₄₅), and for comparison made of
16 PLA, TCPS (tissue culture polystyrene) as well as RG756 (a trade mark for poly(D,L-
17 lactide-co-glycolide 75:25), were poured out on polypropylene discs. The bone
18 marrow stem cells of 6 week old male Sprague Dawley rats with a concentration of
19 5000 cells per cm³ were cultured onto these films. After 3 hours the morphology of
20 the adhered cells was then observed with the scanning electron microscope.

21 The results obtained are shown in FIG. 8. The number of cells was
22 additionally determined by counting using the optical microscope. It was evident that
23 the number of cells on the block copolymer according to the disclosure was less, the
24 larger the hydrophilic component b) of the polymer. Moreover, the images taken by
25 scanning electron microscope showed that any cells which had adhered to the block
26 copolymer according to the disclosure were in some cases more rounded than on the
27 reference polymers comprising only hydrophobic constituents, which is a clear sign
28 for the low adhesion tendency of the cells to the polymer surface.

29

30

EXAMPLE 6

1 Characterization of the active polymers with respect to their binding capabilities.

2 6a) Identification of the binding capability with simple model substances with
3 amino group in solution

4 For examination of the reactivity in solution, a specific amount of polymer
5 (SWS-NH-PEG₂-PLA₂₀) (50 mg) was dissolved in 2000 µl of dimethylformamide
6 (DMF) and mixed with a specific amount of dye (EDANS, 5-((2-
7 aminoethyl)amino)naphthalene-1-sulphonic acid, sodium salt, 0.1-4 mg) which was
8 also dissolved in DMF. In order to exclude any possible protonation of the amino
9 group, 20 µl of triethylamine were added as proton catcher. The solution thus
10 obtained was then incubated overnight in the agitator at 37°C. After the reaction
11 period, 200 µl of the solution were then diluted with 1800 µl of chloroform and the
12 excess precipitated dye was separated by filtration. 200 µl of the clear solutions were
13 then measured by means of gel-permeation chromatography. The amount of
14 covalently bonded dye was determined via the increase in uv absorption at 335 nm.

15 The result is shown in FIG. 9. If the surfaces obtained are evaluated, then a
16 diagram is obtained in which an increase in peak surface may be observed as the
17 amount of dye increases. From a specific amount of dye a plateau is then obtained
18 which is also determined by the restricted number of reactive groups. The amount of
19 reactive groups in a batch of polymer may be simply determined via this
20 determination.

21 6b) Identification of the binding capability with simple model substances with
22 amino group on solid polymer surfaces.

23 The activity on solid surfaces may be examined just as the activity in solution.
24 For this, films of an active block copolymer according to the disclosure (SWS-NH-
25 PEG₂-PLA₂₀), which had been poured onto round glass cover plates, were coated with
26 an aqueous solution of the dye (5-amino eosin) and this solution was then left to work
27 for two hours. The marked films thus obtained were washed with phosphate buffer
28 several times and then dried. The dried films were then dissolved in chloroform and
29 then separated by means of GC possibly adsorbed from covalently bonded dye. The
30 presence of an increased UV absorption was observed with the molecular weight of

1 the polymers. This UV absorption may be explained by a covalent bond between dye
2 and polymer.

3 6c) Binding of proteins.

4 For examination of the binding ability also of more complex compounds such
5 as proteins, the enzyme trypsin was used as model substance.

6 To bind the enzyme to polymer films, films of the various polymers (SWS-
7 NH-PEG₂-PLA₂₀ with PLA for comparison) poured onto glass cover plates were
8 incubated with solutions of the enzyme trypsin in phosphate-buffered isotonic
9 common salt solution (PBS buffer). The concentrations of the enzyme used for this
10 amounted to 0.5 or 1.0 mg/ml.

11 The polymers linked with trypsin thus obtained, after an incubation period of
12 2 hours, were then washed 3 times with PBS buffer containing 0.05% Tween 20 in
13 order to remove any possibly adsorbed protein as effectively as possible. The films
14 thus washed were then wiped dry and transferred into six-well plates. 2 ml of the
15 reaction medium were then added to each individual well of the plates and the
16 enzymatic reaction was conducted in the incubator for 2 hours at 37°C. The reaction
17 medium was a 1 millimolar solution of benzoyl-L-arginine ethyl ester (BAFE) in tris-
18 buffer with pH=8.0. After 2 hours the enzymatic reaction was stopped by adding an
19 aqueous solution of a trypsin inhibitor composed of soya beans and the transformation
20 of the enzyme substrate was thus terminated. The solutions thus obtained were
21 measured at 253 nm by uv-photometric means.

22 The result is shown in FIG. 10. The comparison with PLA and with the pure
23 glass cover glasses shows a clear increase in the substrate conversion in the case of the
24 block copolymer according to the disclosure which is caused by the amount of
25 covalently bonded enzyme.